



Influence of tree rejuvenation, IPNM and VA-Mycorrhizal fungi on shoot emergence, yield and fruit quality of *Psidium guajava* under farmers field condition

K. K. Chandra^{1*}, S. K. Pandey², Ajay K. Singh¹

¹Department of Forestry, Wildlife and Environmental Sciences, Guru Ghasidas Vishwavidyalaya, Bilaspur, Chhattisgarh 495009, India

²Indira Gandhi National Open University, Regional office, Darbhanga, Bihar, India

Received: 14 September 2012

Revised: 25 October 2012

Accepted: 26 October 2012

Key words: Rejuvenation, VAM, Root infection, IPNM, BCR.

Abstract

The present study was conducted during 2007 to 2010 in 15 year old guava orchards at Chandauli (U.P.) India. The selected trees were headed back (Rejuvenated) from 1.5 - 2.0m height from ground level applied IPNM, VAM fungi alone and in combination just after rejuvenation and to compare the response from unrejuvenated tree practiced by farmers of guava var. *Allahabadi safeda* L. There was 56.44% gap in technology adoption and farmers practice resulted in low yield with poor fruit quality. The rejuvenated guava tree applied IPNM and VAM was found to be most effective enhancing emergence of new shoots (3.76 fold) fruit weight (1.89 fold) and TSS (1.11fold). The fruit yield recorded 140.55% higher in trees with IPNM and VAM followed by IPNM and VAM over the yield of control. The nutrients content in different treatment were also found significantly higher compared to control. The VAM fungi significantly increased the uptake of N, P, Mg, Zn, Cu and Fe over control. The root infection was examined 66.5% and 72.55% in trees inoculated with IPNM+VAM and VAM respectively and 13.5% in control without inoculation. BCR of FP was followed decreasing trend while BCR of rejuvenated trees with IPNM and VAM was in increasing trend. The cost of rejuvenation of guava tree observed higher and yield was lower in initial year results lower BCR but just in 3 years of rejuvenation the BCR increased 2.37 fold, 2.12 fold and 2 fold with IPNM+VAM, IPNM and VAM respectively than control plot.

*Corresponding Author: K. K. Chandra ✉ kkchandra_31@rediffmail.com

Introduction

Guava (*Psidium guajava* L.) is known as poor man apple found in market of over 60 countries throughout the tropics and subtropics including some Mediterranean areas. It exceeds majority of other fruits in adoptability, productivity, grown in wide range of soil and climate all over the world relished for flavor, delicious taste and high nutritive value give more than one crop during the year. It is richest source of vitamin C, calcium, fair in phosphorus and iron. It is well adopted in almost all states of India but the most productive area is indogangatic plain extending from west to east of Uttar Pradesh and Bihar orcharding commercially in horti-olericulture agroforestry model and in homestead garden. The guava contributes 4 percent of total fruit production with acreage of 0.25 million ha with annual productivity of about 7 t ha⁻¹ (Pilania et al., 2010). Indian farmers don't fertilize their trees and careless on management results in low productivity. The declining yield pattern from old guava orchard over the years is the major cause shifting the interest of farmers towards other crops and cropping system.

There are various attributes limiting production and productivity of guava which pertinent and gravest for declining trends. The majority of orchards became old and senile characterized by intermingling, overcrowded, infestation of insect and disease in branches and trunk, more wood mass and thin shoots in canopy adversely influences bearing quality fruits (Singh and Singh, 2003). The fruits of guava are borne on new wood 9-11 months old and any treatments that encourage new growth influence the fruiting directly. In addition, researches are established that the fruiting potential of guava is largely governed by canopy architecture, density and photosynthetic efficiency (Burondkan et al., 2000, Kallo et al., 2005). The Indian farmers are advocated for rejuvenation of old senile orchards to allow new shoots on tree, elimination of infected branches, increase light penetration on floor for field crops along with higher fruit yield. This technology play significant role in conversion of old

orchard into new once but it requires at most care, skilled felling, scheduled irrigation and adequate nutrition. So far the technology is not becoming popular only because some trees in orchard sprouts poorly, appear sickly and few start dyeing after following rejuvenation techniques due to inadequate nutrition and water management.

Integrated Nutrient management comprises organic, inorganic and microorganisms are highly beneficial for sustainable food and fruit production as it ameliorate soil environment, maintain adequate level of nutrients and provide favorable conditions for higher yield with divine quality (Law-Ogboma and Egharevba, 2009, Hiwale et al., 2010). The positive effect of organic, chemical fertilizers and biofertilizers (azotobacter, PSB) has been also confirmed by Madhavi et al. (2008) in mango, guava and sapota fruits. Like other free living biofertilizers, vesicular arbuscular mycorrhizal fungi (VAM) also known for improving the growth, development and yield of plants by increasing the absorptive capacity through establishing symbiotic relationship with most of terrestrial plants including horticultural tree species (Guissou, 2009) vegetables (Ortas, 2010). Interestingly, it work more efficiently in harsh condition such as nutrient deficiency (Miller and Jastrow, 2000), drought (Khalaffallah and Abo-Ghalia, 2008) and disease and insect incidences (St-Arnaud and Elsen, 2005) and helps host plants to withstand and grow under adverse conditions.

VAM is ubiquitous in nature reported widely in soil and plant species belongs to agriculture, forestry and horticulture but its contribution on emergence of new shoots, fruit quality and yield and economics is not evaluated so far in guava. Therefore the present study was conducted to assess the influence of INM and VAM fungi in rejuvenated guava trees in farmers field and also analyze the gap between improved technology and actual adopted technology.

Material and methods

Study site

A demonstrative experiment on VA-Mycorrhizal fungi and integrated nutrient management was carried out at farmers field by Krishi Vigyan Kendra (Narendra Deva University of Agriculture and Technology, Kumarganj, Faizabad), Chandauli (U.P., India) located in 25.27°N and 83.27°East during three consecutive years (2007-08, 2008-09 and 2009-10). The average precipitation in the area during study period recorded 1025.40mm yr⁻¹, while the maximum temperature noticed 43°C in May and minimum 8°C in January month. Soil of the experimental field was sandy loam, pH 7.3 with 0.67 % organic matter, N-288 ppm., P- 3.60 (mg kg⁻¹), K- 350 (mg kg⁻¹), Zn- 0.61 (mg kg⁻¹), Cu-1.67 (mg kg⁻¹) and 135 spores of VAM in soil g⁻¹⁰⁰.

Determination of technological gap

Prior to conduct present study a group meeting was organized inviting 100 guava farmers of Rema, Digghi, Ganjkhawaja, Faguiya and Tajpur villages (Guava growing villages) to identify the root cause problem of low yield as well as to identify the technological gaps between improved production technology and actual farmers practice. Finally five farmers were selected among groups of farmer randomly for field experiment.

The gap in use of practice was calculated by following formula:

$$M.T.G. = \left(\frac{TGP}{TPC} \right) \times 100\%$$

MTG= Mean Technological Gap, TGP= Total Gap for all practices, TPC= Total No. of Practice Considered .

Experimental plan and module of treatment:

Five farmers selected and convinced to provide 48 guava trees for heading back (12 from each farmer) to maintain four replications (4 tree replication⁻¹) in randomized block design.

Fifteen year old sick and decline trees were marked and headed back to 1.5 m to 2.00 m height above ground level during May 2007 to allow the

emergence of new shoots and development of fresh canopy of healthy shoots. The newly emerged shoots were again pruned from about 40 to 50 cm length in October for emergence of multiple shoots. To check rainy crop shoots pruned from 50 percent of its length again in May. Further management of shoot was continued in 2008 and 2009 during May and October months for proper umbrella shaped canopy development and to ensure production of quality fruit. Pasting of copper oxichloride on cut surface and painting of tree trunk with copper and lime were followed after each pruning.

The trees were irrigated at regular interval by flooding the basins around the tree except during the rainy seasons to maintain moisture for proper growth of shoot and fruiting twig. After six month of rejuvenation IPNM package (25 kg vermicompost + 1 kg neem cake + 1300 g urea 1800 g (46% N), single super phosphate (16% P₂O₅), 500 g muriate of potash (46% K₂O) plant⁻¹) and 50g micronutrient mixture was applied in two split doses in the month of October and June. The treatment details are given below.

T₁ = Rejuvenation (Headed back) + Pruning of emerging shoots + IPNM package + VAM (*Glomus intraradices* and *Acaulospora scrobiculata*) 400 g inoculum (Density 10³ infective propagules with roots and spores)

T₂ = Rejuvenation (Headed back) + Pruning of emerging shoots + IPNM package

T₃ = Rejuvenation (Headed back) +VAM (*Glomus intraradices* and *Acaulospora scrobiculata*) 400 g inoculum (Density 10³ infective propagules with roots and spores)

T₄ = Farmers practice (No head back of tree but application of DAP (18% N and 46% P₂O₅) @ 300 g plant⁻¹yr⁻¹)

The compost, inorganic fertilizers and VAM inoculum were broadcasted 30 cm from the trunk and out to the edge of canopy of the tree. Data on tree growth was recorded in the month of October every year and flowering and fruiting parameters

were recorded after shoot pruning in May and October month. Fruit size and other quality parameters were determined using a 10 fruit sample from each tree by standard laboratory procedures, from random samples of 40 fruits from each plot. Total soluble solids were measured by Erma hand refractometer. Percent increase in yield was calculated by following formula.

$$\text{P.I.Y.} = \left(\frac{Y_2 - Y_1}{Y_1} \right) \times 100\%$$

PIY= % increase in yield, Y₂= Yield of treated tree,

Y₁ = Yield of Control tree

The yield was expressed as the total number and weight of fruits produced per tree in each season harvested in August and December. Twenty leaves of uniform size and color from the third pair from shoots of current season's growth were collected to form a composite sample and determined the nutrient elements in leaves. Total N was analyzed by kjeldahl procedure, P by spectrophotometry, K by using flame photometer and micronutrients by AAS following standard procedures recommended by Jackson (1973). To identify the VAM fungal colonization rate, randomly sampled roots were collected from treated trees and cut into at least 1 cm long fragments and stained at 0.5% trypan blue (Phillips and Hayman, 1970). The stained root pieces were examined under compound microscope and expressed as a percentage of total root length colonized. Clamydospores of VAM fungi were screened from soil as per Gerdemann and Nicolson (1993) and counted under stereo-microscope. For each fruit tree species, all data were statistically analyzed by the procedure of analysis of variance using SPSS software.

Results and discussion

The technological gaps in Guava farming was assessed and tabulated in table 1 revealed that the farmers of this region did not adopt recommended cultivation practices therefore obtaining low yield and income from the guava farming. Overall gap in guava farming estimated 56.44 percent whereas gap in nutrient management noticed 56%. It was

noticed that over 78% respondents did not aware about pruning and training and use of biofertilizer besides other improved technologies. It was also noticed that commercial growers did not intercrop in between orchard while marginal and small cultivars utilized the space of tree for growing vegetables, elephant foot yam and turmeric crops for the additional income. More or less no gap was found in planting techniques and in selection of varieties. The farmers were using unbalance dose of fertilizer since long time in growing guava and still unknown to use about IPNM practices due poor economic conditions. Javaria and Khan (2011) also reported that small land holding, poor socioeconomic condition and low level of education of farmers are the root cause constraints for technological extension in developing country.

Data shown in Table 2 indicate significant treatment differences in guava trees rejuvenated by headed back and treated with IPNM and VAM singly or in-combination. Tree rejuvenation with regular pruning decreased the tree height up to 50% compared to unrejuvenated trees (Fig. 1). This initiated emergence of new shoots and stimulated shoots to convert in flowering shoots. It was clearly seen from the parameters measured in the present study than the application of IPNM with VAM inoculation was found to be most effective among other treatments at $P < 0.05$. The shoot emergence increase 3.7 fold in rejuvenated tree followed with IPNM and VAM fungi compared to tree without rejuvenation. The treatment of IPNM and VAM without combination but regular pruning observed beneficial in shoot emergence (Fig.2), flowering and fruiting. The yield increment was found maximum 140.55% with combined application of IPNM and VAM followed by IPNM (89.17%) and VAM (56.50%) over control. The marked effect of IPNM and VAM on yield might be due to the cumulative effect of rejuvenation, nutrition and its better absorption through VAM fungi which reflected on new shoot emergence, canopy development, flowering and fruiting as also ascribed by Pilania et al. (2010) and Mitra et al. (2010) in

meadow orchard. The rejuvenation caused branching complexity resulted more fruiting shoots, profuse flowering in these shoots were also reported by Compbell and Wasielewski (2000). In addition Guissou (2009) noticed that VA-Mycorrhized plants absorbed more nutrients from the soil results increase in growth and biomass. This was an agreement with our present finding that VAM and IPNM applied tree rendered more shoots, flower and fruit. The growth of terminal and lateral shoots was stimulated by rejuvenation technique therefore initial yield was adversely affected on such trees but later year the yield increased 3.76 fold due to higher number of fruits and higher weight than control (Fig. 3). This was also observed that the tree require high amount of nutrition just after heading back for faster recovery of canopy, probably root become more active at this stage and tree interacts with mycorrhiza for efficient absorption of nutrients which was proved with the strong root infection in inoculated trees (Fig. 4).



Fig. 1. Rejuvenated Guava orchard.



Fig. 2. Emergence of new shoots

The fruit quality expressed in terms of fruit weight and TSS was recorded significantly higher in all treatments of the present study. The maximum fruit size was obtained IPNM and VAM fungi which gave 1.89 fold higher fruit size than control. The number of fruits were higher with increased weight resulted in to higher due to increased nutrients uptake via VAM fungi. Hiwale et al. (2010) also reported similar results in guava and in Sapota.

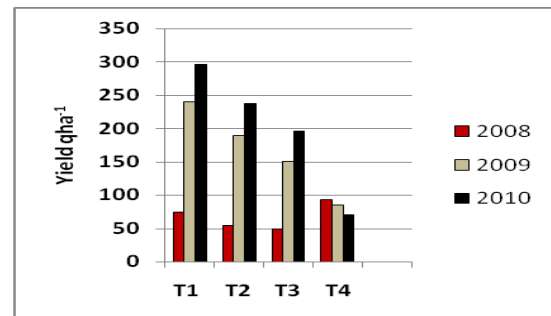


Fig. 3. Fruit yield of rejuvenated Guava trees applied with IPNM and AMF fungi under field condition.

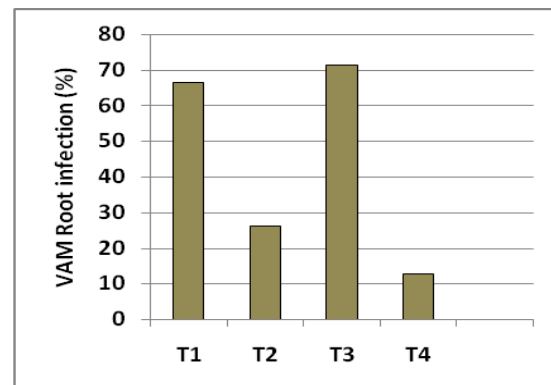


Fig. 4. VAM root infection in rejuvenated guava tree influenced by Mycorrhizal inoculation and IPNM under farmers field condition.

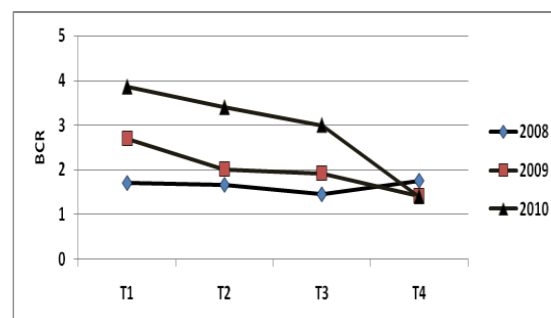


Fig. 5. Benefit cost ratio (BCR) of guava rejuvenated and treated with AMF and IPNS under field condition.

Table 1. Mean technological gap between recommended technologies and farmer practice of Guava farmers.

S. N.	Technologies	Recommended Technology	Farmers Practice	Mean Technological Gap (%)	Reason for non adoption
1.	Varieties	Improved varieties - Allahabad safeda, Lucknow 49, Lalit etc.	Insists only for grafted sapling	14.35	Unawareness and unavailability
2.	Planting	Planting at 6X6 m spacing	Planting at 6X6m spacing (277 pl ha ⁻¹)	00.00	-
3.	Nutrient management	INM includes FYM 20kg + 250g <i>azotobacter</i> +300:200:350g N: P ₂ O ₅ :K ₂ O tree ⁻¹ in two split doses. Mycorrhizal inoculation 400 g tree ⁻¹ or application of PSB culture @ 200 g tree ⁻¹	300g Di-ammonium phosphate tree-1	56.00	Unawareness and unavailability
4.	Mulching	Mulch with paddy straw, banana leaf, black polythene	Not in practice	98.00	Unawareness
5.	Pruning and training technology	Mild pruning in April in fruiting plants and rejuvenation in senile old orchard	Not in practice	98.00	Unawareness
6.	Intercropping	Okra, Cowpea, turmeric, elephant foot yam	Rice and wheat	67.00	Unawareness
7.	Pest control	IPM need based Control of fruit fly by use of Methyleugenol pheromone trap and spray of carbaryl, management of anthracnose with hiophenol 0.01% and Wilt by <i>Trichoderma</i> and <i>Aspergillus niger</i>	No control measures applied	55.00	Unawareness and unavailability
8.	Crop Regulation	Spray Urea 10–15% for deblossoming of rainy season crop.	Not in practice	78.00	Unawareness
9.	Use of bioregulator	Spray of NAA @100ppm or GA ₃ @150 ppm or ethephane @ 300ppm to increase fruit setting and reduce fruit drop.	Not in Use	92.63	Unawareness
Overall gap in technology				56.44	

Table 2. Effect of Mycorrhizal inoculation and IPNM on growth, flowering and yield of rejuvenated Guava cv. *Allahabad safeda*. (Data in parenthesis shown standard deviation of 4 replicates).

Treat ment	Tree height (m)	Emergen ce of new shoot (no.)	Flowering shoots (%)	Mean Fruit Yield (kg tree ⁻¹)	Percent increase in yield over FP	Quality parameter		
						No. of Fruit pl ⁻¹	Fruit weight (g fruit ⁻¹)	TSS (brix°)
T1	3.75 (0.108)	11.80 (1.394)	59.00 (3.740)	73.44 (3.498)	140.55 (6.517)	410 (6.329)	176.50 (11.142)	13.00 (0.707)
T2	3.80 (0.216)	10.05 (0.521)	45.32 (3.905)	58.00 (3.544)	89.97 (9.487)	355 (10.801)	166.00 (7.118)	13.00 (0.294)
T3	3.50 (0.216)	6.33 (0.270)	32.00 (3.265)	47.78 (4.709)	56.50 (9.475)	353 (5.165)	130.40 (5.746)	12.00 (0.248)
T4	7.4 (0.629)	3.15 (0.324)	15.66 (1.491)	30.53 (4.217)	-	300 (11.602)	93.33 (2.649)	11.00 (0.355)
CD (p=0.05)	1.35	0.77	3.28	4.00	9.25	7.35	8.50	0.88

Table 3. Effect of VA-Mycorrhizal inoculation and IPNM on nutrient uptake in rejuvenated and non rejuvenated guava trees. (Data in parenthesis shown standard deviation of 4 replicates).

Treat ment	N %	P %	K %	Mg ppm	Cu Ppm	Zn		
						Ppm	Fe Ppm	Mn Ppm
T1	1.69 (0.155)	0.19 (0.021)	1.58 (0.147)	0.76 (0.028)	16.50 (1.870)	230.00 (21.602)	143.00 (5.614)	144 (11.575)
T2	1.50 (0.217)	0.15 (0.016)	1.54 (0.329)	0.49 (0.069)	13.39 (0.795)	180.00 (22.730)	125.00 (3.560)	139 (4.898)
T3	1.44 (0.077)	0.15 (0.035)	1.25 (0.041)	0.49 (0.063)	13.41 (1.821)	181.50 (7.430)	121.00 (2.160)	130 (5.715)
T4	1.05 (0.131)	0.09 (0.014)	1.20 (0.243)	0.33 (0.021)	11.36 (1.160)	165.00 (26.920)	111.00 (2.160)	124 (6.133)
CD (p=0.05)	0.18	0.03	0.17	0.10	0.60	11.22	3.15	7.40

The nutrient content in guava leaves found significantly higher in present study (Table 3). There was higher N (60.95%), P (111.1%), K (31.66%), Mg (130.3%), Cu (45.24%), Zn (39.39%), Fe (28.82%) and Mn (16.12%) in tree applied with IPNM and VAM compared to the control. Inoculation of VAM found beneficial in enhancing the nutrient particularly P (66.66%), N (37.14%) and Mg (48.48%) over control as compared to other elements. Mathur and Vyas (2000), Dutta et al. (2009) and Pilania et al. (2010) also confirms the benefit of VAM and other biofertilizers and IPNM in nutrient uptake and yield. The root infection was examined 66.5% and 72.75% in inoculated treatments which was 5 fold and 5.59 fold higher than control depicts higher benefits of VAM to plant (Figure 4). There are similar researches conducted by Dutta et al. (2009), Guissou (2009) and Ortas (2010) displaying the positive role of VAM on host growth and yield of several horticultural crops.

Tree with farmers practice exhibited lowest BCR with decline trends while the tree with IPNM and VAM found increasing trend with increased age of rejuvenated tree (Fig. 5). The cost of rejuvenation and IPNM initially caused for low BCR but in later year it was increased 2.37 fold in IPNM+VAM plot and 2 fold in IPNM plots due to quality fruits with higher quantity compared to control.

In conclusion, our results clearly showed that tree rejuvenation followed by IPNM and VAM was found highly beneficial for emergence of new shoots, flowering and improving the fruit quality and yield. VAM fungi promote plant growth and yield especially at stage such as rejuvenation when tree needs high nutrition by playing important role in shoot stimulation flowering and fruit formation. The farmer's perception may be altered by demonstrating such technology in their fields for better adoption, because they are poor in education and income hence needs practical demonstration of such technology to improve the productivity and socioeconomic condition of the farmers. In addition awareness should be developed among farmers to

promote IPNM and biofertilizer application to conserve soil fertility and increase productivity by ecofriendly methods.

References

Burondkan MM, Rajput JC, Waghmare GM. 2000. Recurrent flowering: A new physiological disorders in 'Alphanso' Mango. *Acta Horticulture* **509**, 669- 673.

Compbell RJ, Wasielewaski J. 2000. Mango tree training for the hot tropics. *Acta Horticulture* **509**, 641- 651.

Dutta P, Maji SB, Das BC. 2009. Studies on the response of biofertilizers on growth and productivity of Guava. *Indian J. Horticulture* **66** (1), 39- 42.

Gerdemann JW, Nicolson TH. 1993. Spore of mycorrhizal endogone species extracted from soil by wet sieving and decanting technique. *Trans.Br Mycol. Soc* **46**, 235- 244.

Gonogle MC, Miller TP, Evans MH, Fairchild DG, Swan JA. 1990. A new method which give an objective measure of colonization of roots by *vesicular arbuscular mycorrhizal* fungi. *New Phytol.* **115**, 495- 501.

Guisso T. 2009. Contribution of arbuscular mycorrhizal fungi to growth and nutrient uptake by jujube and tamarind seedlings in a phosphate deficient soil. *African Journal of Microbiological Research* **3(5)**, 297- 304.

Hiwale SS, Apparao VV, Dandhar DG, Bagle, BG. 2010. Effect of nutrient replenishment through organic fertilizers in Sapota. *Indian J. Horticultur* **67(2)**, 274- 276.

Jackson ML. 1973. Soil chemical analysis, Prentice Hall of India, New Delhi, p. 498.

- Javaria SK, Qasim M.** 2011. Impact of nutrient management on tomato yield and quality and soil environment. *J. Plant Nutrition* **34**, 140- 149.
- Kallo, G, Reddy, BMC, Singh, G, Lal, B.** 2005. Rejuvenation of old and senile orchard. Pub. CISH, Lucknow, p. 40.
- Khalafallah AA, Abo-Ghalia HH.** 2008. Effect of *Arbuscular Mycorrhizal Fungi* on the metabolic products and activity of antioxidant system in wheat plants subjected to short term water stress followed by recovery of different growth stages. *J. Appl. Sci. Res.* **4(5)**, 559- 569.
- Law-Ogboma KE, Egharevba RKA.** 2009. Effect of planting density and NPK fertilizer application on yield and yield component of tomato in forest location. *World Journal of Agri. Sci.* **5(2)**, 152- 158.
- Madhavi A, Maheswar PV, Girwani A.** 2008. INM in Mango. *Orissa Journal of Horticulture* **36(1)**, 64- 68.
- Mathur N, Vyas A.** 2000. Influence of arbuscular mycorrhizae on biomass production, nutrient uptake and physiological changes in *Ziziphus mauritiana* under water stress. *J. Arid Environ.* **45**, 191- 195.
- Miller RH, Jastrow JD.** 2000. In. Mycorrhizal fungi influence soil structure. In Kapulniky, Douds Jr. DD. (ed.) *Arbuscular mycorrhiza: physiology and function*. Kluwer Academic Publication, p. 3- 18.
- Mitra, SK, Gurung, MR, Pathak, PK.** 2010. Integrated nutrient management in high density guava orchards. *Acta. Horti (ISHS)* **849**, 349- 356.
- Ortas I.** 2010. Effect of mycorrhiza application on plant growth and nutrient uptake in cucumber production under field conditions. *Spanish Journal of Agricultural Research* **8(SI)**, 116- 122.
- Philips JM, Hayman DS.** 1970. Improved procedures for clearing and staining parasitic and vesicular arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans. British Mycological Soc.* **55**, 158- 161.
- Pilania S, Shukla AK, Mahaver LN, Sharma R, Bairwa HL.** 2010. Standardization of pruning intensity and integrated nutrient management in meadow orcharding of Guava. *Indian Journal of Agriculture Science* **80(5)**, 673- 678.
- Singh G.** 2005. High density planting of guava, application of canopy architecture. *ICAR, News (April-June)* **11 (2)**, 9- 10.
- Singh VK, Singh G.** 2003. Strategic approaches of precision technology for improvement of fruit production. In *Precision farming in horticulture*; Singh, H.P.; Singh, Gorakh; Samuel S.C. and Pathak, R.K. (ed.). NCPAH, DAC, MOA, PFDC, CISH, Lucknow, p. 75- 91.
- St-Arnaud M, Elsen A.** 2005. Interactions with soil borne pathogens and nonpathogenic rhizosphere microorganism. In Declerck, S, strullu, DG, Fortin JA (ed) *Root organ culture of mycorrhizal fungi*, New York, Springer Verlag, pp 217- 231.